OBSERVATIONS ON THE FUNCTIONING OF THE ALIMENTARY SYSTEM OF THE SNAIL LYMNAEA STAGNALIS APPRESSA SAY

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Introduction

Although records exist of functional studies on the alimentary system of Basommatophora as far back as the early eighteen hundreds, the detailed story of the course and ultimate fate of food in the alimentary tract and the simultaneous movements of the tract is thinly scattered and far from complete. In the more recent emphasis placed on some gastropods because of their importance as vectors of parasites of man, domestic animals, wild game, and fish, it is vitally important that the normal physiology of the system most frequented by these parasites be better known.

It is the purpose of this paper to integrate the previous work on the physiology of the alimentary system of *Lymnaea stagnalis* and allied forms (suborder Basommatophora, order Pulmonata) with original research on the same system in *L. s. appressa* Say. The basic morphological (Carriker, 1945) and histological (Carriker and Bilstad, 1946) studies on this system in *L. s. appressa* have been completed and are in press. All terms used in this research have been described in these two papers.

L. s. appressa has been selected for this research because it is a representative vector and because of its excellent response to laboratory culture, its relatively large size (maximum shell length, 62.5 mm.) as compared with other fresh water pulmonates, its short life cycle, and its relatively thin semitransparent shell and semitransparent tissues. Snails used in the research were cultured entirely in the laboratory. They were grown through many generations in large battery jars and fed on lettuce and cooked "cream of wheat" cereal. The water in the jars was aerated by means of a small Marco air pump (Noland and Carriker, 1946). The original snails were collected in Fox Lake, Wisconsin, in 1939. Parasite-free cultures (especially of trematodes) from the original snails were obtained by the isolation of the egg mass soon after oviposition in separate aquaria. Each new culture was started in this way rendering transmission of infection very improbable. Detailed examination of succeeding generations has not disclosed parasites.

This work was carried out at the University of Wisconsin (1939–1943) under the stimulating guidance of Prof. L. E. Noland, whose advice, encouragement, and friendly cooperation were much appreciated.

Historical Review

Scanty observations on the function of the anterior part of the alimentary tract of *Lymnaea* were given by Semper (1857), Geddes (1879) and Moquin-Tandon (1885); more detailed information was given by Amaudrut (1898), Pieron (1908)

and by Baecker (1932). The stomach region was investigated by Gartenauer (1875), Moquin-Tandon (1885), Colton (1908) and Heidermanns (1924). These experimental contributions of Colton and of Heidermanns, particularly of the latter, are noteworthy. The liver has been the object of most of the physiological work although the research has usually been incidental to that on the stylommatophoran *Helix:* Barfurth (1880, 1881, 1883a and b), Frenzel (1886), Cuénot (1892), Enriques (1901, 1902), Faust (1920), Peczenik (1925) and Krijgsman (1928). Only the investigation of Peczenik is exclusively on *L. stagnalis*.

Experimental Methods and Results

Lymnaea physiological salt solution

The study of the living system has required the development of a physiological salt solution which will approximate the ionic and osmotic balances of the blood of *Lymnaea* more closely than do such commonly used solutions as Ringer's. On the basis of incomplete data given by Duval (1928) on *Lymnaea* and by Bernard and Bonnet (1930) on *Helix* on the molecular concentration of blood, the following solution was developed for *L. s. appressa:*

NaCl	2.0 gms. per liter		
NaHCO₃	2.0 " " "
KH₂PO₄	0.1 " " "
MgCl₂	0.3 " " "
CaCl₂	0.3 " " "

This solution consists of 0.47 per cent salts and gives a pH of approximately 7.8. After about a week considerable precipitation of $CaCO_3$ occurs, although this seems to have no noticeable effect on the isolated organs. The vas deferens was used in testing the solution and was found superior to the heart for this purpose. The vas deferens, terminal preputium and prostate gland were removed under the physiological salt solution from the cephalic hemocoel without bruising. This portion of the reproductive tract is in part a strong muscular tube which is easily excised and maintains a continuous squirming motion as long as the tissues are alive. It continued squirming for about 66 hours in the solution described above. A Ringer's solution of 0.7 per cent salts keeps it moving for about 12 hours, although at a much reduced rate.

Hydrogen ion concentration

The first work on the estimation of the pH of the alimentary tract of a fresh water snail seems to be that done by A. H. Rosenbloom on *L. s. appressa* in his bachelor's thesis in 1942 (unpublished) in this laboratory. He has kindly consented to the incorporation of his results in this paper. His method was essentially the colorimetric one employed by Yonge (1925) : fluids from the various lumina of the alimentary tract of the snails under variable feeding conditions were pressed out onto paraffined plates and thoroughly mixed with indicators (brom-thymol blue, neutral red, and methyl red). The colors were compared with those of indicators freshly prepared in buffered solutions checked on a Coleman pH electrometer. The results are given in Table I:

TABLE I

pH of the contents of various lumina of the alimentary tract of L. s. appressa

Organ	Average pH	Maximum and minimum pH	Number of tests	Number of snails
Buccal cavity........	Same as water in external medium.			
Proesophagus........	6.9	7.2–6.3	10	10
Postesophagus........	7.2	7.6–6.6	10	10
Gizzard and crop......	6.4	7.2–6.3	12	12
Pylorus..............	6.6	7.0–5.8	10	10
Intestine.............	7.1	7.8–6.2	35	15

Enzymes

Preliminary tests were made for non-purified cathepsin, pepsin, trypsin, and amylase. The tests for the proteinases were made according to the methods of Anson (1938), Bradley (1938) and Folin and Ciocalteu (1927); those on amylase, by the iodine test of Hawk and Bergeim (1937). Semi-micro technics were applied to large numbers of the excised organs.

Maximum catheptic activity (at pH 3) over a ten-day period was found in the liver. That occurring in the buccal mass and gizzard and other portions of the alimentary system was not significant as compared to that in the liver. In an effort to determine to what extent cathepsin might be secreted from the liver, gut fluid from which the amebocytes had been centrifuged was tested. Under the conditions of the experiment, at least, no cathepsin was found in the gut juice. In some tests tryptic activity was found in the salivary glands. A very active amylase, optimum pH 7, was present in the salivary glands and in the liver.

The only investigation of the hydrolytic enzymes of the alimentary system of the Basommatophora reported in the literature is that by Heidermanns (1924). He described a positive test for cellulase present in the digestive juice of the stomach organs (crop, gizzard, and pylorus) of *L. stagnalis*.

Ciliation

Ciliary currents were studied by the injection of fine carmine suspensions in *Lymnaea* physiological salt solution through various portions of the exposed tract, by application of carmine particles to the epithelium of the opened tract and by placing small bits of gut wall in a carmine suspension on an uncovered microscopic slide under high magnification. In some dissections the undisturbed food particles were seen passing through various portions of the excised gut on the natural ciliary currents.

No work has been performed previously on the ciliation of the alimentary system of the Basommatophora. Merton (1923) in his research on the external ciliation of pulmonates included a brief study of the ciliation of the hepatic ducts of *Helix*.

The entire alimentary system of *L. s. appressa*, with the exception of the gizzard and portions of the buccal cavity, is ciliated (see later in this paper), Figures 3, 9, and 11.

Muscular activity

The activity of the alimentary system was observed under binoculars through the transparent walls of normal living young snails and in adult unanesthetized snails opened under *Lymnaea* physiological salt solution. The independent activity of the radula over the odontophore was clearly observed and conclusively verified by watching snails under the binocular under the following conditions: snails deprived of food for a day were placed in a finger bowl of well aerated water to which had been added strips of lettuce (1–2 mm. wide). A Petri dish was floated over the lettuce and the water. As the snails crawled upside down under the glass, feeding on the lettuce, the action of the radula and mouth parts was clearly visible under a strong beam of light.

Sand in the gizzard

In order to check the experiments of Heidermanns (1924) and to add additional information on the role of sand in the comminution of food by the gizzard of *L. s. appressa,* the following experiments were devised.

Sixteen adult snails were placed in each of four aerated aquaria containing a one-half inch mesh wire platform over the bottom. By means of this contrivance the feces were removed from the vicinity of the snails soon after defecation. To three of the aquaria the following foods were added respectively: (1) cooked "cream of wheat," (2) filter paper, and (3) lettuce. (4) No food was added to the fourth tank. (5) A fifth tank was assembled as a control without the wire platform and with lettuce and sand. One snail from each aquarium was killed daily and opened immediately. After ten days the following was disclosed: eight of the forty-three experimental pulmonates contained no sand in the tract, thereby showing that it is possible to rid completely the tracts of a few of the snails of sand; however, there was extensive variation in the ability of the different snails to retain sand. As the quantity of sand in the gut decreased, the snails consumed less food, until in the absence of sand in the tract, no food was ingested and the guts became void of food material and feces. The different diets indicated no significant difference in their respective values as sand eliminators. Sand was found most abundantly in the gizzard lumen, then in decreasing amounts in the crop and retrocurrent passage of the pylorus (anatomical terminology has been described elsewhere, Carriker, 1945). After the quantity of sand in the lumen of the gizzard reached a certain low level, it was retained with surprising tenacity for many days. The material in the fecal pellets of the control snails, particularly of the gizzard residues, was markedly brown and more thoroughly triturated than those of snails with sand-free diets.

In a second set of experiments snails approximately 10 mm. in length were placed in a one-quarter inch mesh wire basket suspended in a large laboratory snail stock tank. The feces, propelled by the sluggish circulation of the water in the tank, passed out of the basket. All lettuce placed in the basket was carefully washed to remove sand. The experiment was continued for several months. In spite of precautions, small quantities of fine sand were always present in the tracts of some of the animals; however, this did not seem to be enough for proper trituration as many of the snails died abnormally at an early age and none reached the normal adult size of the control snails in the tank outside the experimental basket. There

is unquestionably a vital need for the presence of at least a limited quantity of sand in the gizzard of these snails for sufficient breaking down of the food.

These results are in agreement with the findings of Heidermanns (1924) and of Colton (1908). Heidermanns accidently discovered that the only way to entirely remove the sand from a live snail was to cause it to hibernate, in which state it emitted the total contents of the tract. Colton noted that in the presence of sand the plant food was cut to pieces by *L. columella*, but that in the absence of sand it went unmolested.

Digestive cell ingestion

By the use of a method patterned after that of Peczenik (1925) the ingestion of particulate food by the digestive cells was investigated. White of egg was strained through cheese cloth. Carbon (lamp black) was ground into the egg albumen and the mixture was thoroughly beaten. This was steamed to a stiff mass and fed to snails starved for a few days. After feeding commenced, the snails were opened every other day. Indigestible residues within vacuoles in the digestive cells as well as similar residues in the fecal pellets showed the presence of very minute particles of carbon, particles not present in the control snails. The indigestible residues in the digestive cells appeared very similar to the albumen passing down the intestine in the gizzard residues.

Fecal rhythms

Some information was gathered on the rhythms of the liver and of the gizzard by a study of the rate and extent of passage of the various fecal strings. The fecal pellets of a 40 mm. snail were observed daily for twenty-four days. The animal was isolated in a two-liter glass jar over the bottom of which was placed a paraffined one-half inch mesh galvanized metal screen, so that all fecal pellets fell to the bottom of the jar and could not be reconsumed. The mollusc was fed lettuce on which was sufficient sand for the needs of the stomach region. Three egg masses were oviposited by the snail, and it added 2 mm. of shell during the twenty-four day period. Upon dissection at the end of the experiment the animal appeared normal in all respects. For the first ten days the pellets were collected and examined microscopically every few hours during the day; during the latter part of the experiment they were collected every twelve hours. Numerous examinations were made of fecal pellets from the stock snail tanks to corroborate the findings on the experimental snail.

Physiology of the Alimentary Tract

Buccal mass and esophagus

L. s. appressa is primarily an herbivore. In the laboratory it may complete its life cycle on lettuce alone and in its natural state feeds on the aquatic vegetation of its surroundings. Specialization of the alimentary system (Carriker, 1945) has been in keeping with a plant diet. However, animal food is also consumed as has been observed by Walter (1906) and by seven other authors cited by him. Repeatedly in this laboratory *L. s. appressa* has been observed to eat the bodies out of the

shells of dead snails in the aquaria. Biochemical tests disclose the presence of some tryptic activity in the secretion of the salivary glands.

Pieron (1908) has found in *L. auricularia* and *L. stagnalis* that there is a total absence of food discrimination in the buccal mass and that their feeding is a reflex which keeps the radula working most of the time. The only portion of the body showing any discrimination is the anterior surface of the foot which contains faintly sensitive chemoreceptors. In aquaria in this laboratory *L. s. appressa* rasps much of the time, whether on lettuce or over the newly cleaned glass surface of its tank. However it does also pass through regular "resting" periods in which no rasping occurs. In the rasping stroke the radula passes first to one side and then to the other describing a broad feeding track.

Feeding can be followed clearly in normal immature "albino" *L. s. appressa* (a strain with very little dark pigment) feeding on a "cream of wheat" food mixture blackened with lamp black. This can be seen to pass as far as the stomach region. On the protractor stroke the radula cups to an elongated spoon-shaped trowel about one-half the width of the upper mandible, and working against this, cuts out long narrow bits of food. Each denticle is sharp so the concerted action of the numerous denticles on the radula, sliding independently over the odontophore, provides an effective cutting-rasping apparatus. The food bits are pushed back through the dorsal food channel to the rear of the buccal cavity which dilates to receive them. The tip of the radula closely appresses to the dorsal wall of the buccal cavity in its rearward passage, as attested by the jagged pattern of the dorsal chitinous surface. The buccal aperture constricts strongly and rapidly after the receding radula. Some bits of food are dropped and remain in the dorsal food channel for the next rearward swing of the food-laden radula. Several food bits clump in the rear of the buccal cavity prior to being forced down the esophagus. The radula functions principally in cutting pieces of food of suitably small dimensions for convenient transport through the anterior portion of the alimentary tract; it does not triturate the food to any considerable degree.

Only the posterior third of the buccal cavity is ciliated. These cilia and those in the densely ciliated esophagus beat strongly posteriorly, bearing food bits from the rear of the buccal cavity to the crop.

In connection with the functioning of the buccal mass, refer to a previous paper (Carriker, 1945) for the names, origin, and insertion and relations of the muscles and parts of the mass. The muscular activity of the buccal mass is divisible into four major synchronous movements: (1) opening and closing of the oral aperture and consequent spreading and approximation of the mandibles and lips, as well as dilation and contraction of the circular muscles about the anterior portion of the buccal cavity, (2) backward-forward and simultaneous elevator-depressor movements of the odontophore, with some slight turning of the odontophore on its longitudinal axis and some movement to the right and to the left, (3) movement of the radula and radular sac over the cartilage, and (4) backward-forward and simultaneous elevator-depressor movements of the entire buccal mass. Consequently there exist in the buccal mass three intrinsic focal points about which the majority of the muscles radiate: (1) the oral aperture, (2) the odontophoral cartilage, and (3) the radula and the radular sac.

The activity of the odontophore with respect to the remainder of the buccal mass may be arbitrarily divided into four phases, and described as follows: (1) the *quies-*

cent stage in which the odontophore lies at rest in the rear of the buccal cavity with its longitudinal axis in a dorsoventral position. (2) The *protracting stroke* in which the proximal end of the odontophore swings in an arc of about 130° from its basal position to a point where it lies above the plane of the distal end, which then is in a position to pass partly out of the buccal cavity, bringing the radula against the substratum. At the beginning of this stroke the odontophore assumes a horizontal position as a result of the lowering of the distal end by contraction of the dorsal odontophoral flexor muscle, and a simultaneous raising of the proximal end by strong contraction of the posterior jugalis muscle. The oral aperture and the anterior portions of the buccal mass dilate to permit partial protrusion of the odontophore through the mouth; the labial retractors, suboral dilators and dorsomandibular dilators spread the mouth. The extrinsic postventral levators and posterior jugalis further raise the rear of the buccal mass so that the distal tip of the odontophore is directed towards the oral aperture, to which it seems to be guided principally by the action of the dorsal odontophoral flexor muscles. The inframedian radular tensors draw the radula over the distal end of the cartilage to the point where most of the radula outside the radular sac lies on the under side of the horizontally inclined cartilage, and the collostylar hood lies just behind the distal crest of the cartilage. The combined action of the radular sac and cartilage tensors holds the radula tautly drawn over the cartilage in readiness for the rasping stroke. Contraction of the intracartilage tensors adds considerably to the rigidity of the cushion under the radula. As Woodward (1895) points out for *Natalina caffra,* the fibers of the cartilage act in much the same way as the intrinsic muscles of the human tongue and in contraction cause an elongation and consequent slight protrusion of the radula. The pressure of the blood in the odontophoral sinus probably provides further turgidity. Contraction of the extrinsic as well as of the intrinsic protractor muscles brings the odontophore to the substratum. (3) In the *rasping stroke* the distal tip of the odontophore is drawn over the substrate in a licking motion. The radula, independent of the principal motion of the cartilage under it, is itself simultaneously slid quickly backward most of its length over the cartilage by the action of the heavy supralateral and supramedian radular tensor muscles. The odontophore is aided by contraction of the extrinsic preventral levator muscles which pull the anteroventral floor of the buccal cavity forward and upward. As the mouth opens during the previous stroke, the cutting distal margin of the dorsal mandible is turned partly forward by contraction of the dorsomandibular dilators and possibly the posterior jugals. Thus as the radula rasps forward it makes connection with and scrapes past the inner side of the dorsal mandible, much as two jaws would come together, so that the snail when feeding on thin portions of lettuce actually "bites" off pieces with each rasping stroke. It is only when feeding on thicker foods that true "rasping" comes into play. The dorsal mandible is governed by the dorsomandibular approximator muscle. The lateral mandibles afford mechanical protection to the sides of the mouth, and close in medially after the radula and under and behind the dorsal mandible. (4) The *retractor stroke* returns the odontophore to the resting condition, and completes the cycle, by action of the extrinsic retractor muscles and the supralateral and supramedian radular tensors and relaxation of the protractors. The oral aperture is closed after the receding odontophore by action of the labial sphincter and the mandibular approximator muscles; the buccal cavity, by a contraction of the buccal sphincter and related mus-

cles of the walls. In assuming the resting position, the radular sac is depressed behind the cartilage and the radula rests principally behind the vertically arranged cartilage so that the ventral tip of the sac projects slightly below the level of the buccal mass. As observed by Amaudrut (1898) for *Lymnaea*, the ventral wall of the buccal cavity between the esophageal ledge and the collostylar hood is also depressed, forming a slight dilation in front of the esophageal opening. As both the oral aperture and the proesophagus are closed during the retractive stroke of the radula, it is likely that this dilation is instrumental in creating a slight vacuum in front of the esophageal opening which aids in disengaging food particles from the radula. The dilation is caused principally by depression of the radular sac and possibly by contraction of the superior suspensor muscle of the radular sac and the hood tensor muscles.

The proesophagus is limited in its muscular activity to slight peristaltic waves proceeding towards the postesophagus; while the latter undergoes pronounced peristaltic activity in either a forward or a backward direction, dilating broadly and contracting its entire length. In dilation it may become so large as to fill much of the cephalic hemocoel of the expanded mollusc. In expansion it is filled with a reddish fluid from the stomach region and food particles.

In the buccal cavity the food receives generous quantities of fluid from the buccal gland cells, a fluid which is probably mostly mucoid in nature, judging from the positive mucicarmine stain and from negative tests for amylase and trypsin. This does not however preclude the possibility of the presence of other enzymes which were not tested for. As food passes under the openings of the salivary ducts it receives mucus, amylase, trypsin, and possibly other enzymes from the salivary glands.

The proesophagus adds more secretion from buccal glands and mucous cells. The postesophagus functions as a temporary reservoir for the retention of food when the crop is full. Being capable of considerable distension, it may retain larger quantities of food than the crop. Digestion begins in the postesophagus because of enzymatic secretions received from the salivary glands.

Stomach region

Comminution of food particles is completed in the crop, gizzard, and anterior portions of the retrocurrent passage of the pylorus. These three organs act as a unit comparable to a grist-mill. The kneading motion of the anterior and posterior gizzard constrictor muscles and the gizzard lobes over the sand in the lumen provides the grinding action. Food bits forced between the sand are soon crushed to minute particles upon which the digestive enzymes may act more efficiently. Two synchronized movements are present in the gizzard. In the first the anterior and posterior gizzard constrictor muscles alternate smoothly in mild contraction, thus mixing and forcing the contents of the gizzard slowly back and forth; in the second, not as frequent as the first, the bulk of the gizzard compressor muscles contract suddenly and strongly, bringing pressure to bear on the contents of the gizzard. The presence of gritty material in the gizzard of the Lymnaeidae has been noted by many: Cuvier (1817), Wetherby (1879), Whitfield (1882), Moquin-Tandon (1885), Colton (1908), F. C. Baker (1900, 1911), and Heidermanns (1924).

In the crop, all ciliary currents lead to the anterior margin of the right gizzard

pad, those on the left side beating ventrad and over to the right (Fig. 3). Thus
fine food material accumulates on the right side of the crop at the anterior edge of
the right gizzard lobe. The crop receives food from the postesophagus and forces
it into the gizzard lumen. When ample sand is accessible to the animal, the crop
and anterior portions of the retrocurrent pyloric passage are both filled with it.
The walls of these organs act as mechanical obstructions to the open ends of the
gizzard lumen and concentrate the pressure of the gizzard musculature upon the
contents. They also cooperate in the muscular activity of the gizzard in a unified
kneading and a slow rotation of the gritty contents. The retrocurrent passage re-
turns to the crop those particles which have been dislodged from the gizzard con-
tents by muscular movements of the stomach region. In this fashion the contents
of the gizzard undergo thorough comminution and partial digestion before the resi-
dues are shunted down the procurrent passage to the prointestine.

The epithelium of the stomach region bears a complicated system of ciliary cur-
rents (Figs. 1, 2, 3, 9). Cilia in the procurrent passage direct fine particles from
the right ventral side of the gizzard cavity to the prointestine. Those in the retro-
current passage are directed anteriad towards the left side of the gizzard cavity.
The dorsal passage bears what in fixed sections appears to be nothing more than a
brush border. Even in carmine suspensions under high magnification no distinct
current could be detected in it. The cilia on the ventral fold are divided into two
distinct functional areas: those on the right half of the fold beat obliquely posteriad
and laterally in the direction of the currents in the procurrent passage; those on the
left half, obliquely anterolaterad in the direction of the gizzard and the currents in
the retrocurrent passage. The currents on the minor fold whip obliquely antero-
laterad; those on the medial half of the major fold pass obliquely anterolaterad;
while those on the lateral half of the major fold and those on the medial half of the
fold adjacent the hepatic vestibule reach posterolaterad. The ciliary currents in the
retrocurrent passage are noticeably faster than those in the procurrent passage.
Currents on the atrial corrugations run into the incurrent tubule of the cecum.
Thus the pylorus in cross section (Fig. 2) is composed of three channels, each with
distinct ciliary currents and of three folds which almost meet centrally and whose

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EXPLANATION OF PLATE I

(All figures concern *L. s. appressa*)

FIGURE 1. Stereogram of the pylorus, hepatic vestibule, atrium, cecum, anterior portion of
prointestine, and liver lobes. The vascularization is stressed. (Small arrows indicate the flow
of blood in the arteries; large arrows, the direction of movement of the contents of this part of
the tract.) × 6.

FIGURE 2. Stereogram of cross-section of the pylorus, taken midway between the gizzard
and the hepatic vestibule. The stippled surfaces are heavily ciliated. (The small arrows in-
dicate the direction of the ciliary beat; the large arrows, the direction of passage of material in
the pylorus. The arrows with broken stems designate the direction of ciliary beat on surfaces
behind the folds.) × 25.

ABBREVIATIONS

AT, atrial artery; *CC*, cecal artery; *d.p.p.*, dorsal pyloric passage; *GD*, dorsogastric artery;
HN, minor hepatic artery; *HP*, prohepatic artery; *IP*, prointestinal artery; *m.p.f.*, major pyloric
fold; *n.p.f.*, minor pyloric fold; *pc.p.*, procurrent pyloric passage; *PM*, major pyloric artery;
PN, minor pyloric artery; *PP*, propyloric artery; *PV*, ventropyloric artery; *rc.p.*, retrocurrent
pyloric passage; *v.p.f.*, ventropyloric fold; *VT*, vestibular vascular arborescence.

PLATE I
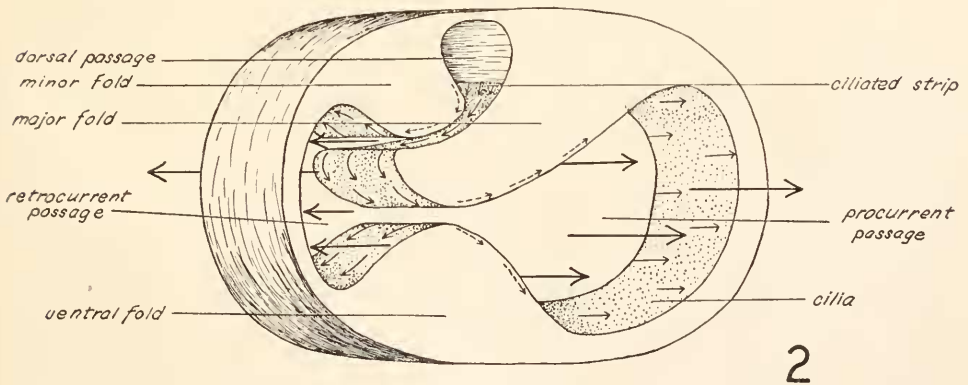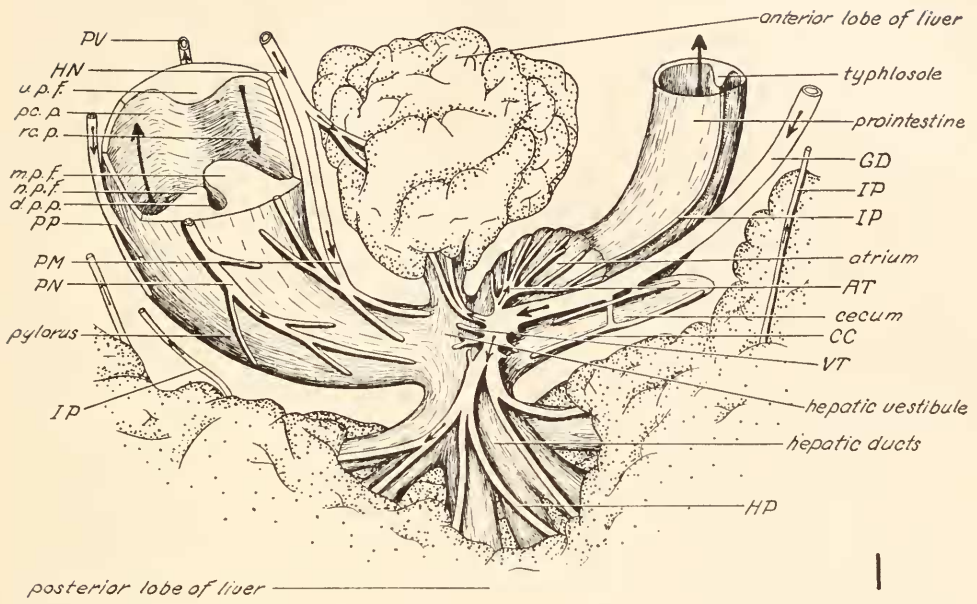
ciliary currents pass out of the dorsal into both the procurrent and the retrocurrent passages. The major fold in addition bears a thin longitudinal strip of long cilia at its boundary with the dorsal passage. The major and minor folds in the living animal nearly always touch along their crests, so that the fluid contents of the dorsal passage may pass into the two ventral passages but coarse material from the ventral passage may not pass into the dorsal passage. The juxtaposition of the two folds is continued under the hepatic vestibule, where the folds provide a ventral floor to this chamber. At this point the cilia on the folds direct a powerful current out and away from the vestibule, again preventing the entrance of coarse material into the hepatic ducts and liver.

As discovered for *Helix* by Merton (1923), the corrugations of the larger proximal portions of the hepatic ducts of *L. s. appressa* bear two ciliary countercurrents (Fig. 11) : the cilia on the crests of the corrugations are long and beat into the liver, those in the grooves are shorter and pass particles in the direction of the hepatic vestibule and into the incurrent tubule of the cecum. The particles in the grooves are quickly entrapped in mucus secreted there and formed into delicate strings. The currents directed into the liver could be traced with certainty only in the large hepatic ducts, although cilia were observed as far as the peripheral follicles in isolated bits of living liver tissue. Yonge (1936) states that in Mollusca where food passes into the liver and waste material out, the ducts are ciliated in such a way that an inward passage exists on one side and an outward one, on the other. Such counter currents could not be determined in *L. s. appressa*.

In the cecum the cilia on the cecal folds beat off the folds into the tubules (Fig. 9) ; those in the incurrent tubule pass carmine particles directly to the distal end and around this into the excurrent tubule. Here the cilia beat circumferentially, rotating the contents of the tubule along the longitudinal axis. In the continuation of the excurrent tubule across the pyloric wall the ciliary stream is directed towards the prointestine.

The crop, pylorus, liver, and hepatic ducts are as active as the postesophagus. Besides the usual peristaltic movements, they undergo a series of violent alternating pulsations, here designated *pulsatory movements*, in which the crop, pylorus, hepatic ducts, and liver pulsate successively, forcing the fluid contents slowly back and forth in swirling currents. In the pylorus the pulsations commence at a point between the typhlosole and the atrium and pass towards the gizzard. They are of two types: (1) very strong pulsations in which the entire structure contracts and (2) minor pulsations running over restricted portions of the pylorus. In the liver the pulsations pass as far as the terminal follicles. This marked movement is most vividly observed in bits of living liver tissue under high magnification. Individual cells are seen to move against each other by contraction of the thin muscular connective sheet enveloping each follicle. The pylorus undergoes the most pronounced movements and appears to lead the other organs in activity. The incurrent tubule of the cecum is relatively thin-walled and does not appear to undergo peristaltic activity. The excurrent tubule is thicker-walled and has definite peristaltic movement in the direction of the outlet.

It follows then that one of the important functions of the pylorus is that of a filter chamber, separating the digested and the fine, partly digested food particles from the gross material and sand. This is the conclusion which Heidermanns (1924) also reached when he stated that most of the time sand and gross material

are kept from passing into the liver by the pyloric folds. The major and minor folds remain in close approximation along their crests, leaving a narrow slit between the dorsal and the ventral passages which may be called the *pyloric filter*. The cilia on the folds are well developed and beat away from the dorsal passage. During the pulsatory movements of the stomach region only the finest particles and fluid material are permitted ingress to the liver through this filter. The *pulsatory currents*, as these in the gut lumen may be named, are relatively strong and in their streaming between the sand particles and foot bits in the gizzard cavity dislodge large particles of food. Those which are carried into the pro- and retrocurrent passages and which are too large to pass through the pyloric filter, become entangled in the ciliary currents of the folds and are carried quickly back to the *left* side of the gizzard lumen by way of the retrocurrent passage. The particles carried into the crop on the forward streaming of the contents are soon entangled in the ciliary currents of the crop and conveyed to the *right* side of the gizzard lumen. Here, then, is a delicate adjustment by which the larger particles dislodged from the gizzard contents are equally redistributed for further grinding within the gizzard.

At certain intervals during the day the pulsatory movements appear to cease and a portion of the residual material and sand in the gizzard pass out through the procurrent passage to the prointestine. The propulsion of *gizzard strings* (Fig. 10), as these residues may be named, through the procurrent passage is very slow and mostly by cilia supplemented by slight peristalsis. Cilia were found active throughout all portions of the alimentary tract whenever opened; no cessation of ciliary activity (as occurs in some lamellibranchs during increase of CO_2 concentration) or reversal of beating was observed. During emission of the gizzard string, the large portion of the ventral pyloric fold which partly occludes the gizzard lumen flattens to enlarge the opening. As suggested by Howells (1942) for *Aplysia,* it appears that the shape and position of the pyloric folds in *L. s. appressa* are partly maintained by blood pressure in the sinuses.

To what extent digestion does occur in the postesophagus, crop, gizzard, and pylorus is questionable. As amylase from the liver and from the salivary glands, trypsin from the salivary glands and cellulase, at least, are present in the gut contents, some food may be partly hydrolized. Part of the remaining available food is reduced mechanically to particles small enough for ingestion by the digestive cells of the liver. The amebocytes of the gut also appear to aid in digestion. According to Heidermanns (1924) fats and carbohydrates are absorbed in the pylorus by the ciliated cells.

The pyloric filter permits only minute food particles to pass into the liver. Most of the radular teeth which are discarded continuously from the radula throughout the life of the snail (Carriker, 1943a) and grains of sand as large as 90 μ, by reason of the fact that they are considerably heavier than the lighter food particles of the same dimensions, are carried past the cilia by the force of the pulsatory currents. The larger free food particles, especially of lettuce, are very light and are readily barred by the cilia of the filter. In the proximal portions of the hepatic ducts, because of counter ciliary currents, only the finer particles that fall into the grooves of the corrugations can be carried towards the cecum; thus teeth and larger sand grains are held at this point by the ciliary currents of the crests of the corrugations until sufficient fecal material passes out of the liver to carry them with it.

Ciliation of the crests of the corrugations may play a small role in the conduction

of food material into the follicles of the liver, but probably the principal conveyers are the pulsatory currents. Food in solution and in suspension is thus brought to all the internal surfaces of the liver follicles. Larger particles finding entrance through the filter and too large to remain readily in suspension appear to fall to the ductal epithelium. The smaller of these are soon propelled into the grooves of the corrugations. Liberal quantities of mucus are secreted there, trapping the particles in mucous strings which pass towards the cecum, coalescing as they advance into the larger grooves (Fig. 11). From the incurrent cecal tubule the mucoid strings pass around the distal end of the cecum into the excurrent tubule. There the material receives a further transparent layer of mucoid and cementing material and is rotated into a smooth cylindrical continuous string, here designated the *cecal string* (Fig. 10). This, partly by ciliary action and partly by peristalsis, then passes on into the prointestine across the atrium. In snails feeding on green lettuce the strings are a vivid green because of a heavy accumulation of bits of chlorophyll bearing bodies which become entangled in mucous strings in the hepatic ducts. In gastropods fed on a food containing carbon, the cecal strings are a dense black. In animals on a starvation diet, the cecum continues to pass out cecal strings, just as in the feeding animal, but the strings are a mucoid, transparent, milky-white color and much reduced in diameter. It thus would seem that the function of the grooves in the hepatic corrugations and of the cecum is to collect and eliminate those fine particles which pass through the pyloric filter but which are too large to be engulfed by the digestive cells and which are thus mechanically eliminated by a "supplementary filter." Cecal strings pass out continuously, apparently at the same uniform rate and without apparent interruption. They provide a kind of "time clock" by which the rate of passage of the gizzard strings and the residues from the liver can be compared (Fig. 10).

EXPLANATION OF PLATE II

(All figures concern *L. s. appressa*)

FIGURE 3. Ciliation currents of the postesophagus, crop, gizzard, pylorus, hepatic vestibule, atrium, and anterior portion of prointestine. The tract has been slit ventrally and spread. × 6.

FIGURE 4. Irregular blue-green excretion bodies (in vacuoles) taken from the liver string. × 500.

FIGURE 5. Smooth blue-green, or brown, excretion bodies (in vacuoles) taken from the liver string. × 500.

FIGURE 6. "Signet" excretion body (in vacuole) appearing in the liver strings. × 500.

FIGURE 7. Clear nodules found in the liver strings which when pressed out under the cover slip display their crystalline nature. They dissolve in dilute HCl and seem very similar to the calciferous concretions of the vesicular cells of the connective tissue. × 500.

FIGURE 8. Indigestible residues from digestive cell (in vacuole), found abundantly in liver strings. × 500.

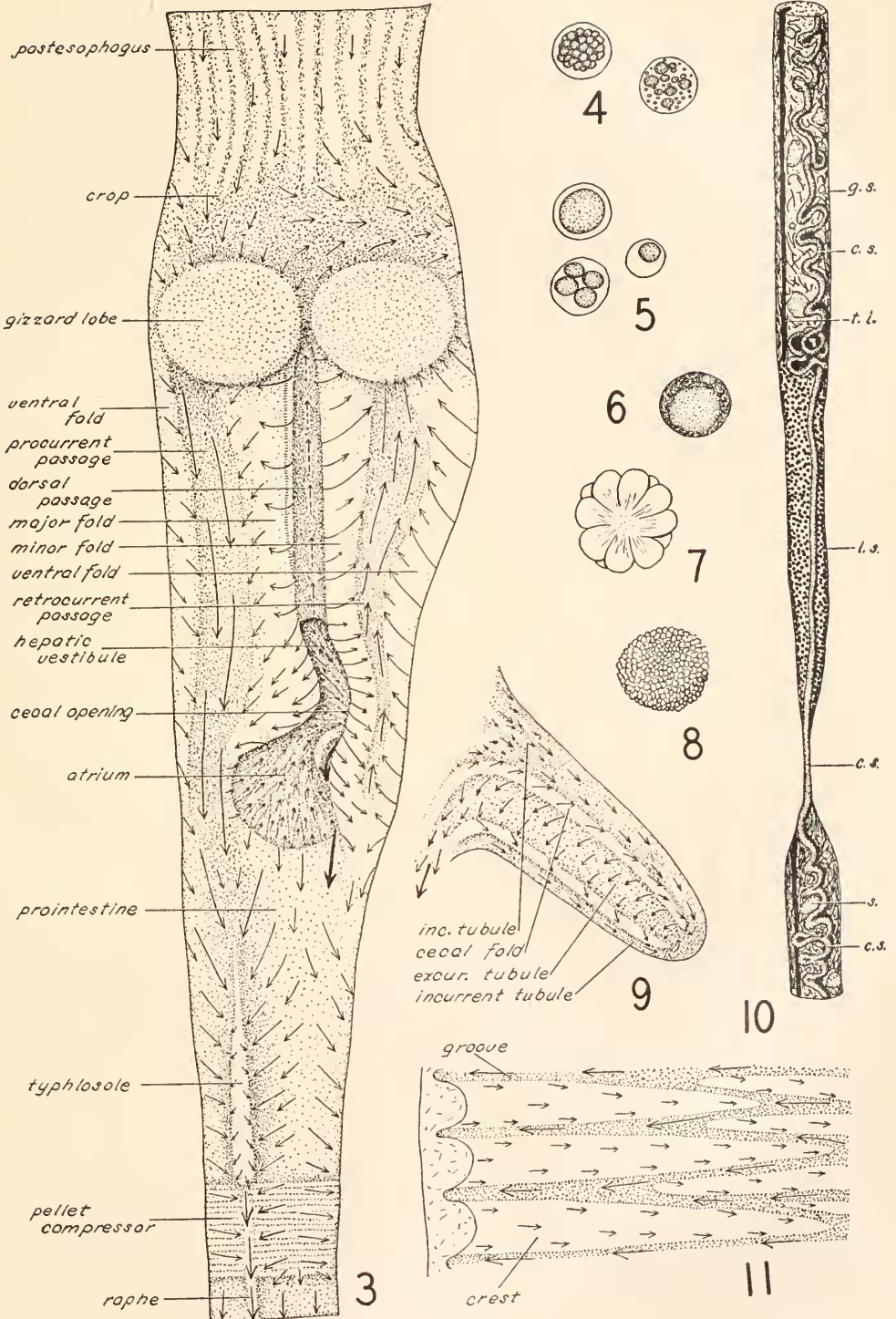FIGURE 9. Ciliation currents of the cecum, which has been opened along the incurrent cecal tubule and spread flat. × 6.

FIGURE 10. Typical fecal pellet, showing the gizzard, liver and cecal strings, and the impression of the typhlosole in the pellet. × 6.

FIGURE 11. Portion of the corrugated epithelium of the hepatic duct, taken at the opening of the duct into the hepatic vestibule. (Large arrows indicate the direction of the ciliary currents in the grooves; the small arrows, that on the crests of the corrugations.) × 50.

ABBREVIATIONS

c.s., cecal string; *excur. tubule*, excurrent tubule; *g.s.*, gizzard string; *inc. tubule*, incurrent tubule; *l.s.*, liver string; *s.*, sand; *t.i.*, impression of typhlosole in fecal pellet.

PLATE II

postesophogus

crop

gizzard lobe

ventral
fold
procurrent
passage
dorsal
passage
major fold
minor fold
ventral fold
retrocurrent
passage
hepatic
vestibule

ceoal opening

atrium

prointestine

typhlosole

pellet
compressor

raphe

3

4

5

6

7

8

9

inc. tubule
cecal fold
excur. tubule
incurrent tubule

10

g. s.
c. s.
t. l.

l. s.

c. s.
s.
c. s.

11

groove

crest

The excretory bodies and indigestible residues in the liver are voided periodically. These are passed simultaneously in minute mucous strings from all parts of the liver towards the central hepatic ducts, there converging into larger strings which pass in the direction of the hepatic vestibule. At the proximal end of the hepatic ducts this material fills most of the main duct. The combined currents in the grooves of the corrugations appear to exert a stronger force than those on the crests, so forcing the waste material directly into the hepatic vestibule (Fig. 11). There it is caught by the outward flowing ciliary currents on the major and minor pyloric folds and passed rapidly into the prointestine. The excretory bodies and indigestible residues passing from both lobes of the liver are compressed in the hepatic vestibule into one bulky string which is distinct from the cecal and from the gizzard string and may be called the *liver string* (Fig. 10). It is drawn out of the liver at the same rate as the cecal string passes out of the ceum. Both strings are usually found parallel to each other and uncoiled in the fecal pellets. The gizzard string, on the other hand, passes out much more slowly so that the cecal string occurs loosely and abundantly coiled therein (Fig. 10). A lapse of time seems to occur between the exit of the gizzard string and that of the liver string, as indicated by a conspicuous coiling of the cecal string between the last portion of the gizzard string and the forward end of the liver string. The gizzard string follows the liver string immediately, as indicated by no noticeable coiling of the cecal string between the two. There is also some evidence that, as the liver string is drawn from the liver, the pulsations of the stomach region cease. In animals opened for physiological observation of the tract, the stomach region was never pulsating when the liver strings were passing out of the liver. This is desirable to prevent the dismemberment of the strings and their mixing with food material brought into the liver by the pulsatory currents. The merger of the strings in the prointestine produces the fecal pellets.

The pylorus is composed of a complicated system of folds and passages, it is innervated by a pair of complex nerve plexuses and a nerve net, and all of the parts are exceptionally well vascularized. Functionally there is present in this portion of the tract an intricate system of counter ciliary currents and synchronized muscular movements, as well as partial vascular control of the folds. The pylorus is thus well equipped to convey digestive fluids from the liver to the gizzard and crop, to bear digested and semi-digested particles into the liver from the gizzard, to exclude large sand and other large particulate matter from the liver and transfer such residues to the prointestine, to receive waste material from the liver and transport it to the intestine, to act in conjunction with the cecum, liver, and hepatic ducts in shunting a continuous string of residual particles from the walls of these organs into the prointestine, to secrete fluids (of unknown nature) and finally to absorb fats and carbohydrates.

Liver

The liver is probably the most important organ of digestion in the alimentary system of the gastropods. Peczenik (1925) shows, as has been indicated in this work also in feeding experiments, that such proteins as egg albumen are engulfed and digested intracellularly in the digestive cells, and the indigestible residues are cast out in vacuoles. Krijgsman (1928) believes that digestive cells in *Lymnaea*

are also secretory as well as absorptive, as he has often observed numerous typical secretion granules in the liver cells of starved snails. Biochemical tests indicate that the greatest catheptic activity of the snail body is localized in the liver, yet none of this activity has been found in the fluid of the gut. This is in keeping with catheptic systems in other animals in which the enzyme has been shown to exist entirely as an intracellular protease. Hurst (1927) writes that in *Physa* fat and glycogen are stored in the digestive cells. Fat was also found in the lime cells of *Helix* by Grünbaum (1913). The problem of *what size* of food particle is engulfed through the distal membrane of the digestive cells is still an open question. It is likely, as indicated by the work of Krijgsman (1925, 1928) on *Helix,* that the lime cells function in storing and in periodically secreting a buffering agent which adjusts the pH of the gut juice; this point has not been investigated in *L. s. appressa.* The mucous cells of the liver provide the mucus utilized in the binding of the indigestible residues and the excretory bodies into the liver strings.

Amebocytes were found in varying numbers in the contents of the lumina of the liver, postesophagus, gizzard, and pylorus. These were similar to those seen in the blood. In some instances those in the gut contained fecal vacuoles so large as to force the cell into a peripheral lobate ring.

Rhythmic activity of the liver is suggested by inspection of sectioned liver tissue, of fecal pellets and of the living organ in various phases of its activity. Pulsatory movements of the stomach region are apparently interrupted only during the passage of liver strings and of gizzard strings. This may explain why smaller hepatic excretory bodies occur in the upper pylorus, gizzard, crop, and postesophagus in such insignificant numbers. If the pulsatory currents persisted during the elimination of the liver residues one would expect to find liver string detritus scattered over the gut in as great profusion as in the liver, along with the reddish colored secretions from the liver.

The inclusion bodies of the digestive cells of *L. s. appressa* have been studied in detail in the living cells of normally feeding snails, starved snails, snails fed on special diets and in preserved tissue sections. The egested bodies have been followed in the fecal pellets over a period of weeks. The results of the study clearly indicate the presence in the digestive cells of excretion bodies, of indigestible residues and of secretion in separate vacuoles.

Figure 8 illustrates a vacuole from the digestive cells which is filled with indigestible particles. These vacuoles measure 12 to 25 μ in diameter. In snails feeding on lettuce the contents are colored a greenish brown to dark brown and are composed of minute irregular particles, some of the larger ones of which measure about 3 μ in diameter. In the digestive cells they occur one per cell and in varying stages of particulate concentration. These constitute the bulk of the liver strings and retain their identity in fecal pellets which have been voided for several days.

The secretion granules are clearly evident in preserved histological sections stained with iron hematoxylin, especially grouped towards the distal area of the cell. Larger granules measure as much as 4 μ in diameter.

The excretion vacuoles (Figs. 4, 5, 6) when in the cells may measure as much as 25 μ in diameter, but in the fecal pellets have shrunk somewhat. In the living cells excretion bodies are found in variable form and color and are best observed when the cells are slowly pressed out under a cover slip as the fluids evaporate. The cell contents then pass rolling and turning from the ruptured cells, exposing the

different surfaces of the inclusions. There is one series in which the vacuoles range from small to large vacuoles containing variable numbers and sizes of minute blue-green, translucent, many-angled particles. The smaller particles are in constant Brownian movement, dancing around like a swarm of bees, and indicating the low viscosity of the fluids in the vacuoles (Fig. 4). In a second series the same variation in size of the vacuole is encountered but the blue-green bodies are present in groups of only one to four per vacuole and are spherical and smooth (Fig. 5). In a third series the vacuoles and bodies are identical in form to the second series, but the color of the bodies varies from a light brown to a dark solid brown. The largest of these bodies are sometimes found free of the vacuoles. When compressed under a cover slip they spread with a flowing viscous movement, much as a drop of heavy molasses spreads when pressed between two smooth surfaces. In the fecal pellets these vacuoles are usually found varying in diameter from 3 to 15 μ, and the vacuole membrane presses closely around the excretion body. A fourth type of excretion body is found which varies in diameter from 12 to 18 μ, is colored a dark brown with a smooth center and possesses a periphery of irregular markings, such that the body resembles a signet ring (Fig. 6). The excretion bodies described above are present principally in the liver strings, and only in negligible numbers in the cecal strings. The "browns" and "signets," particularly, stain with methylene blue and neutral red and do not dissolve in strong HCl. The different types described are not all present in any liver string in equal abundance at any one time, but vary independently, in a sequence which did not seem significant. Because of the transitional stages between some of these excretion bodies it is probable that they are all different phases of the same type of metabolic excretion; but the method of their formation is still a puzzle.

Intestine and rectum

Cilia on the typhlosole beat towards the lateral sides of the typhlosole (Fig. 3); those over the prointestine around the typhlosole beat circumferentially and somewhat obliquely from the dorsal to the ventral sides in a symmetrical pattern. The division of the currents occurs along the dorsal line of the prointestine. Over the pellet-compressor the cilia beat transversely across the intestine. The raphe bears a strong current which streams directly posteriad. Thus in the pellet-forming region, through ciliation and muscular movement, loose particles are gathered, rolled inward about the typhlosole and folded into a compact pellet. Strong ciliary currents in the remainder of the intestine and rectum are limited almost entirely to the costae, raphe, and pseudoraphe; cilia of the intercostal surfaces are relatively short and weak. Peristaltic activity is evident throughout the intestine and rectum, being noticeably strongest in the early portions of the prointestine, just behind the pellet-compressor.

Abundant vascularization of the prointestine, in contrast to the relatively poor vascularization of the esophagus, suggests that this region of the intestine may also function in the absorption of food and water.

Consolidation of the cecal and liver strings occurs at the hepatic vestibule; of the gizzard residues and cecal string, in the pellet-forming region. The cecal string as it is moulded in the cecum is already a smooth well cemented string and undergoes no further change as it is forced continuously across the outer margin of the atrium.

The liver string, characterized by a fine dark brown mottling and almost as well concentrated as the cecal string, receives a final transparent envelope of cementing fluid which binds the cecal string to it (Fig. 10).

The chief function of the pellet-forming region is that of consolidating and cementing the loose straggling gizzard residues which constitute by far the greatest bulk of the fecal pellet. The large numbers of mucous cells, basophilic flask cells and basal secreting cells about the pellet-forming region are indicative of the large quantities of cementing substance secreted during the moulding of the pellets. By means of ciliary streams and constriction of the tube at the pellet-forming region the gizzard residues are pressed into pellets, and the cecal strings, lying loosely
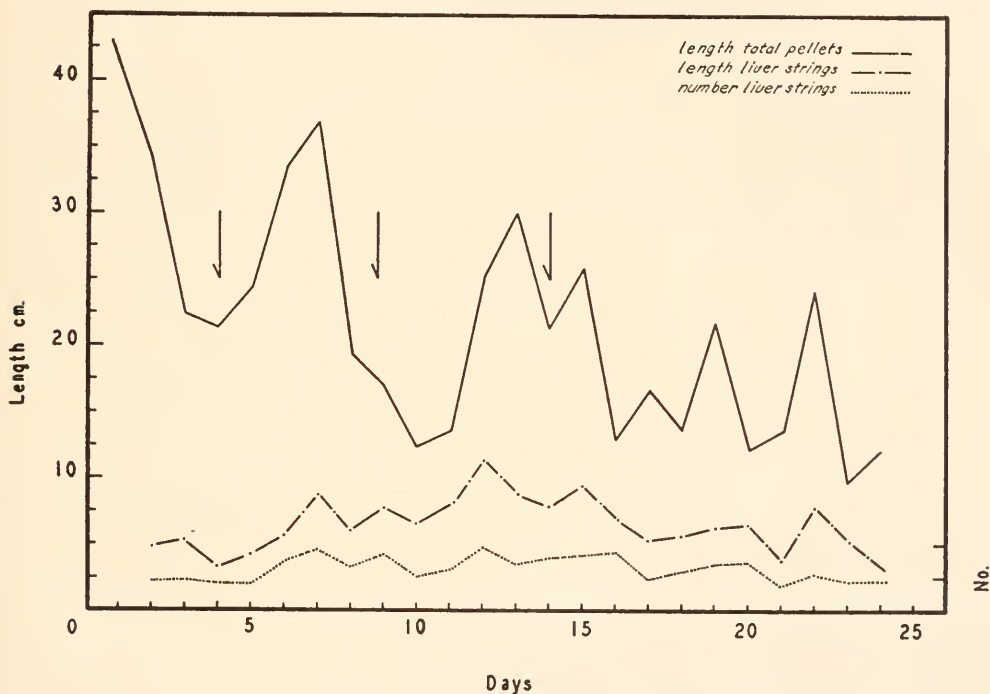

FIGURE 1. Length in millimeters of the liver and gizzard strings and number of liver strings of the fecal pellets, calculated on a twenty-four hour basis. These were voided in a period of twenty-four days by a forty millimeter *L. s. appressa*. The vertical arrows indicate the time at which egg masses were oviposited.

coiled in these residues, are simultaneously incorporated in the pellets. These are then forced out of the pellet-forming region by ciliary activity and by strong peristaltic movements which are noticeably stronger immediately behind the pellet-compressor. Peristaltic activity gradually diminishes in the direction of the anus. The conspicuous impression of the typhlosole remains in the fecal pellet, particularly in the gizzard string portion, as long after defecation as the pellet retains its form. Moore (1931) has found variable patterns in the fecal pellets of different Gastropoda and points out the importance of identification of animals by means of their pellets. A most striking fact about fecal pellet formation is the extreme com-

pleteness with which fecal material is compressed and cemented. This presumably prevents fouling of any portion of the tract.

For any given snail the diameter of the gizzard string portion of the fecal pellet is constant, varying principally with the size of the snail. The liver string varies in diameter from that of the gizzard string to that of the fecal string. Figure 1 indicates for a forty millimeter snail over a period of twenty-four days the rate and extent of voidance of fecal pellets. For the tabulation of this data the fecal pellets were collected daily and arranged end to end under the binoculars and measured to the nearest millimeter. The measurements given indicate only the lengths of the gizzard and liver strings, as the cecal string generally occurs embedded in the first two strings. The diameter of the gizzard string is reliably constant; that of the liver, less so.

Most conspicuous is the fact that the quantity of fecal pellets voided daily is quite variable from day to day. The quantity of gizzard strings fluctuates far more erratically than does that of the liver strings, indicating that the volume of material utilized by the liver is more constant than that which may pass through the gizzard. The number of liver strings is a more conservative indicator than the length of strings, and is probably not as accurate. Passage of food through the gizzard, and thus food consumption, seems to diminish during oviposition.

As indicated by the following data, feces were voided in about equal quantity day and night, with just a slight daily increase, over a period of twenty days (9 P.M. to 9 A.M., and 9 A.M. to 9 P.M., respectively):

Pellets	Night	Day
Total length of pellets, mm.	1,987	2,134
Total length of liver strings, mm.	530	588
Total number of liver strings	110	113

The total length of fecal pellets passed in the twenty-four days was 5,645 mm.; and the total length of liver strings, 1,491 mm., was passed in 289 liver strings, giving an average length of 5.1 mm. per liver string. Actually the liver strings varied in length from one to 10 mm. The average calculated length of fecal pellets passed in twenty-four hours was 235 mm.; of liver strings, 62 mm. In a normally feeding snail the sequence of the liver strings with the gizzard strings was always one of alternation. Liver strings do not generally mix with the gizzard strings. Gizzard strings as long as 52 mm. were found connecting liver strings. Three typical series of fecal pellets taken from days one, two, and three on Figure 1 are given below. The liver and gizzard strings are represented by the lengths in millimeters of the strings in the order of their elimination; the figures for lengths of the gizzard strings are italicized. The total time for elimination of the pellets is given to the right in parenthesis:

(1) 6 *40* 7 *33* 7 *11* 6 *44* 5   (5 hrs. 15 mins.)
(2)   *48* 7 *52* 8 *13* 4 *50* 5 *38* 6 (10 hrs. 15 mins.)
(3)   *20* 7   8 7 *22* 6 *29* 9 *24* 3 (10 hrs. 30 mins.)

As indicated by the curve for total fecal pellets in Figure 1 and by the lengths of the gizzard strings in the series above, consumption of food appeared to follow an alternating heavy and light cycle.

In snails deprived of food the elimination of the gizzard strings ceased and liver strings then became connected only by slender lengths of cecal strings. When starvation had continued for ten or more days nothing but delicate white cecal strings and a few much reduced liver strings containing metabolic excretion bodies were found in the intestine.

A. H. Rosenbloom (unpublished bachelor's thesis, 1942) by feeding colored food to *L. s. appressa* at different times through a period of a month found that in normally feeding snails of approximately forty millimeters shell length the minimum time for the passage of food from the mouth to the anus was two hours and twenty minutes; in snails previously starved for a week, five hours and fifty minutes. He found also that previously starved snails feed for a longer consecutive time than do normally feeding snails. The present investigation shows clearly that the alimentary system becomes completely emptied of food a few days after starvation commences. Considerably more food and a longer time are required for a starved animal to fill the alimentary tract with food to the point where fecal material is voided than for a normally feeding snail.

The rhythm of passage of liver strings is in keeping with the rhythm of the liver itself in which all digestive cells appear to assimilate food together and discharge indigestible residues simultaneously. This cycle, as indicated by the passage of liver strings, is not completely unvarying, because the number of liver strings discharged daily varied approximately from eight to nineteen. Thus the interval between the discharge of liver residues, probably the time during which the liver was digesting food, varied in this experiment from seventy-five minutes to three hours. It is possible that oviposition (Fig. 1) may account for some of the variability.

There seems to be nothing in the literature concerning fecal cycles in the Gastropoda. Some few scattered observations are reported on the length of the fecal pellets. For example, Heidermanns (1924) writes that a 48 mm. *L. stagnalis* with a 90 mm. intestine, eliminated 120 mm. of feces in 24 hours.

The long intestine is characteristic of the herbivorous snail nutrition of *L. s. appressa*. One of the most striking facts about the functioning of the alimentary system is the meticulous care with which all loose particles are collected and properly disposed of, in this way serving as a highly efficient sanitation system. The fecal pellets receive additional external layers of cementing material as they pass down the length of the intestine and rectum. The pH of the intestine is slightly more alkaline than that in the stomach region. As pointed out by Yonge (1935) mucus is an amphoteric protein whose viscosity is augmented by higher pH, thus more efficient consolidation of the feces occurs. Elimination of the fecal pellets through the anus is a fairly rapid and uniform process. The strong anal sphincter muscle remains tightly contracted except during defecation. Fecal pellets, being slightly heavier than water, settle slowly to the bottom of the aquaria. The marked efficiency of the mucoid coating over the feces is indicated by the extended period after defecation that pellets retain their identity. Thus it would seem that the alimentary system has not only become specialized in the maintenance of hygienic conditions within the system, but also in furthering a healthy external environment.

Fecal pellets are ingested by snails even in the presence of fresh food and the animals appear to derive some nourishment from them. It is to be recalled that the gizzard is not a thoroughly efficient grinding mechanism and in many cases,

particularly in the absence of sufficient fine sand, considerable unused available food passes out in the gizzard strings.

DISCUSSION

The question as to whether the radula slides over the cartilage independent of cartilage activity has been a favorite point of academic controversy with certain malacologists for some time (in Lymnaeidae see Geddes, 1879; Amaudrut, 1898; and Pelseneer, 1935; in the Stenoglossa, a review: Carriker, 1943b). In *L. s. appressa* (and possibly in the majority of snails carefully investigated) there is no question but that the principal activity of the radula is that effected by the action of the cartilage and muscles under it, and a sliding of the total radula over the cartilage independent of the movement of the cartilage.

A study of the movements of the gut in *L. s. appressa* suggests that rather than the presence of different pH in the different portions of the gut, the pH may vary with the rhythms and secretions of the liver, the secretions of the salivary glands, the secretions of the unicellular glands of the gut wall and with feeding. It is quite unlikely that with the constant mixing of the gut contents as a result of the pulsatory movements at certain periods, the pH would vary markedly in the different lumina of the tract at any time. The wide range obtained between the maximum and the minimum pH's and the insignificant variation of the maximum and of the minimum pH's is in keeping with this suggestion. The partial isolation of the intestine from the movements of the stomach region is in keeping with the slightly higher pH found in the intestinal lumen.

The complexity and abundance of nervous tissues about the stomach region suggests a possible nervous control of the movements of the stomach region and of the liver. In its muscular structure there is no doubt that the buccal mass is the most complex organ in the alimentary system; functionally it appears that the region in and about the pylorus is the most intricate. The dense ramifications of blood vessels, the presence of two nerve plexuses, the intricate series of folds and the complicated ciliary streams in this region lend credence to this postulation.

Heidermanns (1924) has opened the question of the function of sand in the basommatophoran gizzard in his comparative study of *Ancylus, Planorbis, Physa, Lymnaea* and certain stylommatophorans. He points out that in land pulmonates the flaring portion of the esophagus is called the stomach, whereas in the aquatic pulmonates the esophagus is normal and the stomach has become differentiated into the crop, gizzard and pylorus. Thus the Stylommatophora have no organs that could properly be homologized with the stomach of the Basommatophora. The gizzard and, with few exceptions, sand in the tract are absent from the land pulmonates. The gizzard, he states, reaches its peak of specialization in *L. stagnalis* and probably rose by reason of the ingestion of sand with food. He observed that in all Basommatophora the gizzard originates in front of the first flexure of the gut, apparently as a muscular band whose primitive function was to dispose of sand masses tending to congest there. This primitive type of gizzard is exemplified by that of *Ancylus* and the intermediate type by that of *Planorbis*. Heidermanns in support of his theory of the origin of the gizzard through a specialization of a primordial portion of undifferentiated gut, attempted to show modification of the gizzard in one snail generation by the use of various diets. As might be anticipated, he got no significant structural changes.

The fact that *Lymnaea* possesses the gizzard grinding mill may explain the observation stressed by Heidermanns that the cellulase of this snail is less active than that of *Helix* which has not developed a gizzard and consequently needs a strong cellulase for the hydrolysis of the cell walls of plant food consumed.

There is striking similarity in the functioning of the alimentary tract of the herbivore *Onchidella celtica,* ably presented by Fretter (1943) in a recent paper, and that of *L. s. appressa.* Perhaps this similarity is not to be wondered at when, as Fretter writes, "Many of the features which the Onchidiidae share with the pulmonates may be attributed to the close origin of the two groups, the similarity of their diet and their air-breathing habit."

Summary

1. A balanced physiological salt solution was developed which maintains contractions of the vas deferens for approximately 66 hours.

2. Cathepsin was found in greatest concentration in the liver and no activity could be ascertained in the gut fluids. Some trypsin was indicated in the salivary glands. Amylase showed greatest activity in the salivary glands and the liver.

3. Muscular activity of the alimentary system involves the manipulation of the mouth parts in the buccal mass, peristalsis in the remainder of the tract, marked pulsatory movements of the postesophagus, crop, pylorus and liver, and a kneading motion of the gizzard. The radula is moved principally by the action of the odontophore but also operates independently of it.

4. The entire alimentary system, with the exception of the gizzard and parts of the buccal cavity, is ciliated. The cilia show definite directional streams which function in propelling food particles, in sorting food and in consolidating fine refuse particles with the aid of mucoid substances.

5. Sand is consumed normally by the snail and is necessary for the proper functioning of the gizzard in the crushing of food particles. Very little trituration is performed by the mouth parts.

6. The pylorus is composed of a complicated system of folds and passages and counter ciliary currents and functions as a filter which permits only the soluble and the finer food particles to pass into the liver. It shunts the undigested residues from the gizzard into the prointestine.

7. In the liver the digestive cells function in secretion, assimilation, intracellular digestion and excretion. The indigestible foods and the excretory products, as variably shaped and colored inclusion bodies, are eliminated in vacuoles.

8. The cecum functions in collecting the finer residues from the liver and forces them in a continuous string into the prointestine.

9. The residual material coming from the gizzard, liver and cecum is characteristic for each organ and is readily identified as distinct in the fecal pellet.

10. The prointestine is specialized in the final consolidation of gizzard, liver and cecal strings with the aid of cementing substances secreted by the basophilic flask cells and the basal cells.

11. The rhythmic nature of the liver is disclosed principally by a study of the fecal pellets.

12. *L. s. appressa* is an herbivore. Food bits are cut away by the radula and swallowed. In the buccal cavity the food receives mucus from the buccal gland

cells, mucous cells and the salivaries and enzymes from the latter. Temporary storage and initial digestion occur in the postesophagus. Digestive fluids pass up from the liver in the pulsatory movements of the stomach region which keep the fluid gut contents in constant circulation. The crop, gizzard and anterior portion of the retrocurrent passage of the pylorus comminute the food. Amebocytes present in the gut contents appear to aid in digestion. Soluble and fine particles of food pass through the pyloric filter into the liver where it is assimilated by the digestive cells. Assimilation also occurs in the pylorus and absorption possibly in the intestine. There is some evidence that the pulsatory movements of the stomach region cease during the passage of the gizzard and the liver strings.

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